भारतीय मानक
Indian Standard

IS 4276 : 2014
(Reaffirmed 2019)

सोयाबीन का तेल — विशिष्टि

(दूसरा पुनरीक्षण)

Soybean Oil — Specification

(Second Revision)

ICS 67.200

भारतीय मानक ब्यूरो
BUREAU OF INDIAN STANDARDS
मानक भवन, 9 बहादुरशाह ज़फर मार्ग, नई दिल्ली-110002
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI-110002
www.bis.org.in www.standardsbis.in

September 2014 **Price Group 4**

Oils and Oilseeds Sectional Committee, FAD 13

FOREWORD

This Indian Standard (Second Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Oils and Oilseeds Sectional Committee had been approved by the Food and Agriculture Divisional Council.

Soybean oil is a vegetable oil extracted from the seeds of the soybean (*Glycine max*). It is one of the most widely consumed cooking oils. As a drying oil, processed soybean oil is also used as a base for printing inks (soy ink) and oil paints.

India is the sixth largest producer of soy oil in the world, accounting for 4 percent of world production. India is also importing soybean oil in large quantities.

This standard was first published in 1967 in order to regulate the quality of refined soybean oil produced in the country from imported soybean oil. Later when the cultivation of soybeans started on a bigger scale in the country and substantial amount of soybean oil was produced indigenously, need was felt to revise this standard to cover the oil based on indigenously available data. The first revision of this Indian Standard was published in 1977 and covered raw soybean oil along with degummed, semi-refined and refined oil. The first revision was later amended to introduce scheme for labelling environment friendly products to be known as ECO Mark at the instance of the Ministry of Environment and Forests (MEF).

This revision was carried out to harmonize the standard with *Food Safety and Standards Act*, 2006 and Regulations framed thereunder and *Vegetable Oils Grading and Marking Rules*, 1955.

In this revision the following major changes have been made:

a) Definition of refined soybean oil has been simplified;
b) The nomenclatures and requirements of the different grades of soybean oil have been changed;
c) Solvent extracted semi refined grade has been removed;
d) Grade used exclusively for industrial purpose has been removed;
e) The requirements of use of food grade solvent hexane and absence of non-edible oils have been removed from oils for industrial uses;
f) The limit of aflatoxin has been prescribed for non-ECO marked edible oils also;
g) Aflatoxin is determined using High Performance Liquid Chromatography (HPLC) and Enzyme Linked Immunosorbent Assay (ELISA) instead of Thin Layer Chromatography (TLC) prescribed earlier;
h) The colour of refined oil is determined using 5¼ inch cell instead of ¼ inch cell prescribed earlier and the limits of colour has been modified to reflect the same;
j) Quantitative limit of phosphorous content has been prescribed and the method of test for the same has been included instead of the qualitative test for presence of phosphorous;
k) Limits of refractive index, iodine value and lead content have been changed to align with *Food Safety and Standards Act*, 2006 and Regulations framed thereunder; and
m) The limit of hexane has been included to align with *Food Safety and Standards* (*Food Product Standards and Food Additives*) *Regulation*, 2011.

In the preparation of this standard, due consideration has been given to *Food Safety and Standards Act*, 2006 and Regulations framed thereunder; *Legal Metrology Act*, 2009 and Rules framed thereunder and the *Essential Commodities Act*, 1955. However, this standard is subject to restrictions imposed under these, wherever applicable.

In reporting the results of a test or analysis made in accordance with this standard, the final value, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

SOYBEAN OIL — SPECIFICATION

(Second Revision)

1 SCOPE

This standard prescribes requirements and methods of sampling and test for soybean oil used for edible purposes and for manufacture of *VANASPATI* and refined oil.

2 REFERENCES

The following standards contain provisions which, through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below.

IS No.	*Title*
548	Methods of sampling and test for oils and fats:
(Part 1) : 1964	Methods of sampling, physical and chemical tests (*revised*)
(Part 2) : 1976	Purity tests (*third revision*)
1070 : 1992	Reagent grade water — Specification (*third revision*)
1448 [P : 21] : 2012/ISO 2719 : 2002	Method of test for petroleum and its products: [P : 21] Determination of flash point — Pensky Martens close cup method (*third revision*)
1699 : 1995	Methods of sampling and test for food colours (*second revision*)
3470 : 2002	Hexane, food grade — Specification (*first revision*)
10142 : 1999	Polystyrene (crystal and high impact) for its safe use in contact with foodstuffs, pharmaceuticals and drinking water — Specification (*first revision*)
10146 : 1982	Specification for polyethylene for its safe use in contact with foodstuffs, pharmaceuticals and drinking water
10151 : 1982	Specification for polyvinyl chloride (PVC) and its copolymers for its safe use in contact with foodstuffs, pharmaceuticals and drinking water
10325 : 2000	Square tins — 15 kg/litre for ghee, *VANASPATI*, edible oils and bakery shortenings — Specification (*second revision*)
10339 : 2000	Ghee, *VANASPATI*, edible oil tins up to 10 kg/litre capacity — Specification (*second revision*)
10910 : 1984	Specification for polypropylene and its copolymers for its safe use in contact with foodstuffs, pharmaceuticals and drinking water
11434 : 1985	Specification for ionomer resins for its safe use in contact with foodstuffs, pharmaceutical and drinking water
11704 : 1986	Specification for ethylene acrylic acid (EAA) copolymers for their safe use in contact with foodstuffs, pharmaceuticals and drinking water
12247 : 1988	Specification for nylon-6 polymer for its safe use in contact with foodstuffs pharmaceuticals and drinking water
12252 : 1987	Specification for polyalkylene terephthalates (PET & PBT) for their safe use in contact with foodstuffs, pharmaceuticals and drinking water
13576 : 1992	Ethylene menthacrylic acid (EMAA) copolymers and terpolymers for its safe use in contact with foodstuffs, pharmaceuticals and drinking water — Specification
13601 : 1993	Ethylene vinyl acetate (EVA) copolymers for its safe use in contact with foodstuffs, pharmaceuticals and drinking water — Specification
IS/ISO 14718 : 1998	Animal feedings stuffs — Determination of aflatoxin B1 content of mixed feeding stuffs — Method using high performance liquid chromatography

3 DEFINITION

For the purpose of this standard, the definitions given in **2** of IS 548 (Part 1) and also the following shall apply.

3.1 Refined Soybean Oil — Refined soybean oil means oil which is obtained by solvent extraction of soybean oil bearing materials and processes involving degumming, neutralization, bleaching, deodorization/ deacidification by physical refining.

4 GRADES

4.1 The material obtained by the process of solvent

extraction shall be of following grades:

a) Refined,
b) Degummed, and
c) Grade I.

4.1.1 Refined soybean oil is suitable for direct edible consumption.

4.1.2 Degummed and Grade I soybean oil are suitable for making *VANASPATI* and refined oil only and not for direct edible consumption.

5 REQUIREMENTS

5.1 Description

The material shall be obtained from good quality soybeans from the plant *Glycine max* (L) Merrill Syn. *Glycine Soja* Sieb and Zucc., fam. Leguminosae by a process of solvent extraction.

5.1.1 Refined oil and Grade I oil shall be obtained from the oleaginous material using solvent hexane conforming to IS 3470.

5.2 The material shall be clear and free from adulterants, sediment, suspended and other foreign matter, separated water, and added colouring and flavouring substances. The material shall have acceptable taste and odour and when tested as prescribed in **20** of IS 548 (Part 1), the peroxide value of the oil shall not exceed 10 milliequivalents peroxide oxygen per kg.

5.2.1 The clarity of the material shall be judged by the absence of turbidity after keeping the filtered sample at 30°C for 24 h.

5.3 Oils shall be free from non-edible oils and adulterants when tested in accordance with **9**, **10, 11, 12, 14, 15, 16** and **18** of IS 548 (Part 2).

5.4 Oil shall not contain aflatoxin, more than 30 µg/kg, when tested by the method prescribed in IS/ISO 14718 or as prescribed in Annex A.

5.5 Metal contaminants and pesticide residues shall not exceed the tolerance limits as prescribed in the Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011.

5.6 Only permitted antioxidants and antioxidant synergists not exceeding the quantities specified against each as prescribed under the Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011, may be used, if required.

5.7 The material shall also comply with the requirements given in Table 1.

5.8 Optional Requirements for ECO-Mark

5.8.1 The product shall conform to the requirements of quality given in **5.1** to **5.7**.

5.8.1.1 The manufacturers shall produce to BIS environmental consent clearance from the concerned State Pollution Control Board as per the norms laid down under the *Water* (*Prevention and Control of*

Table 1 Requirements for Soybean Oil
(*Clause* 5.7)

Sl No.	Characteristic	Requirement for Grade			Method of Test, Ref to
		Refined	Degummed	Grade I	
(1)	(2)	(3)	(4)	(5)	(6)
i)	Moisture and insoluble impurities, percent by mass, *Max*	0.10	0.5	0.5	**5** and **6** of IS 548 (Part 1)
ii)	Colour on the Lovibond scale, expressed as ($Y + 5R$) not deeper than	25[1)]	40[2)]	40[2)]	**13** of IS 548 (Part 1)
iii)	Refractive index at 40ºC	←	1.464 9 to 1.471 0	→	**10** of IS 548 (Part 1)
iv)	Saponification value	←	189 to 195	→	**15** of IS 548 (Part 1)
v)	Iodine value (Wijs)	←	120 to 141	→	**14** of IS 548 (Part 1)
vi)	Acid value, *Max*	0.5	20	20	**7** of IS 548 (Part 1)
vii)	Unsaponifiable matter, percent by mass, *Max*	1.0	1.2	1.5	**8** of IS 548 (Part 1)
viii)	Flash point Pensky-Martens (closed), ºC, *Min*	250	100	100	IS 1448 [P : 21]
ix)	Hexane, ppm, *Max*	5.00	—	—	Annex B
x)	Phosphorous content, ppm, *Max*	12	200	—	Annex C
xi)	Insoluble bromide test		To pass the test		Annex D

[1)] in a 5¼ inch cell.
[2)] in a ¼ inch cell.

Pollution) *Act*, 1974; *Air* (*Prevention and Control of Pollution*) *Act*, 1981; *Water* (*Prevention and Control of Pollution*) *Cess Act*, 1977 respectively, along with the authorization, if required, under the *Environment* (*Protection*) *Act*, 1986, while applying for ECO-Mark.

5.8.1.2 The product shall not contain aflatoxin, more than 5 µg/kg, when tested by the method prescribed in IS/ISO 14718 or as prescribed in Annex A.

5.8.1.3 The product shall not contain any of the toxic metals in excess of the quantities prescribed in Table 2.

Table 2 Limits for Toxic Metals
(*Clause* 5.8.1.3)

Sl No.	Characteristic	Requirement	Method of Test, Ref to
(1)	(2)	(3)	(4)
i)	Lead, mg/kg, *Max*	0.5	**15** of IS 1699
ii)	Arsenic, mg/kg, *Max*	0.5	do
iii)	Cadmium, mg/kg, *Max*	1.0	do
iv)	Mercury (total) mg/kg, *Max*	0.25	do

6 PACKING

6.1 The material shall be supplied in suitable well-closed tin or plastic containers, as agreed to between the purchaser and the supplier. Tin or plastic containers once used, shall not be re-used for packaging of edible oils and fats.

Containers made of plastic materials shall be as per IS 10142 or IS 10146 or IS 10151 or IS 10910 or IS 11434 or IS 11704 or IS 12247 or IS 12252 or IS 13576 or IS 13601.

Containers made of tin shall be as per IS 10325 or IS 10339.

6.1.1 For ECO-Mark, the product shall be packed in such packages which are made from recyclable (that is which can be re-processed to manufacture any useful product) or biodegradable materials.

6.2 Types and grades which are not suitable for direct edible consumption shall not be packed in consumer packs.

7 MARKING

7.1 The containers shall be marked in English or Hindi in *Devnagri* script with the following information:

a) Name, trade name, type and grade of the oil;
b) Name and business particulars of the manufacturer;
c) Net quantity of the contents in the container;
d) Batch number, month and year of manufacture;
e) "free from Argemone Oil";
f) *Nutritional information* — Nutritional information or nutritional facts per 100 g or 100 ml or per serving of the product shall be given on the label containing the following:
   1) energy value in kcal;
   2) the amounts of protein, carbohydrate (specify quantity of sugar) and fat in gram (g) or ml;
   3) the amount of any other nutrient for which a nutrition or health claim is made:

   Provided that where a claim is made regarding the amount or type of fatty acids or the amount of cholesterol, the amount of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids in gram (g) and cholesterol in milligram (mg) shall be declared, and the amount of trans fatty acid in gram (g) shall be declared in addition to the other requirement stipulated above.

g) Any other requirement as stipulated under *Food Safety and Standards Act*, 2006 and Regulations framed thereunder and *Legal Metrology Act*, 2009 and Rules framed thereunder.

7.2 The container of imported edible oil shall also bear the word, "Imported", as prefix to type and grade of oil. Where an article of food is imported into India, the package of food shall also carry the name and complete address of the importer in India.

7.3 In addition in the case of grades which are not suitable for direct edible consumption (*see* **4.1.2**), the following information shall be suitably marked, either printed on the label affixed to the container or lithographed or stencilled thereon with indelible ink, in a type size of not less than 50 mm:

Degummed and Grade I soybean oil: "NOT FOR DIRECT EDIBLE CONSUMPTION"

7.4 The package, label or the advertisement of edible oils and fats shall not use the expressions "Super-Refined", "Extra-Refined", "Micro-Refined", "Double-Refined", "Ultra-Refined", "Anti-Cholesterol", "Cholesterol Fighter", "Soothing to Heart", "Cholesterol Friendly", "Saturated Fat Free" or such other expressions which are an exaggeration of the quality of the product.

7.5 For ECO-Mark the containers shall be marked with the following:

a) List of identified critical ingredients in descending order of quantity, percent by mass,

which shall include 'made from soybean oil';

b) The brief criteria for which the product has been labelled for ECO-Mark; and

c) Shelf life of the product.

7.6 BIS Certification Marking

The product may also be marked with the Standard Mark.

7.6.1 The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

7.7 ECO-Mark

The product may also be marked with the ECO-Mark, the details of which may be obtained from Bureau of Indian Standards.

8 SAMPLING

8.1 Representative samples of the material shall be drawn as given in **3** of IS 548 (Part 1).

ANNEX A

(*Clauses* 5.4 *and* 5.8.1.2)

DETERMINATION OF TOTAL AFLATOXIN BY ELISA

A-1 PRINCIPLE

Antibodies specific to aflatoxins B1, B2 and G1 are immobilized on the filter, and toxin (aflatoxin B1) is labelled with an enzyme (horseradish peroxidase). Binding of toxin-enzyme conjugate by immobilized antibodies is inhibited by addition of free toxin present in the test sample. Bound enzyme catalyses oxidation of substrate to form a blue complex. Development of colour indicates that the test sample contains aflatoxin.

A-2 APPARATUS

A-2.1 Antibody Coated Solid Support

A-2.2 Aflatoxin Enzyme Conjugate

A-2.3 High Speed Blender

A-2.4 Variable 100-1 000 µl Micropipettes

A-2.5 Glass Culture Tubes

A-2.6 Filters

A-2.7 Timer

A-2.8 Silicon Carbide Boiling Chips

A-3 REAGENTS

A-3.1 Wash Solution — Phosphate Buffered Saline Solution — Dissolve 0.23 g $NaH_2PO_4.H_2O$, 1.95 g $K_2HPO_4.3H_2O$, 8.70 g NaCl, 0.125 µl Tween 20 and 10 mg thimerosal in 900 µl H_2O, adjust *p*H to 7.2 and dilute to 1 litre.

A-3.2 Buffer — 0.1 percent Bovine serum albumin in phosphate buffer saline solution containing 0.05 percent thimerosal.

A-3.3 Substrate Solution A, tetramethylbenzidine (TMB), (0.4 g/l H_2O), *p*H 8.3.

A-3.4 Substrate Solution B, hydrogen peroxide (0.02 percent H_2O_2 in 0.13 percent aq. Citric acid solution, *p*H 3.0).

A-3.5 Methanol

A-3.6 Hexane

A-3.7 Chloroform

A-3.8 NaH_2PO_4

A-3.9 K_2HPO_4

A-3.10 NaCl

A-3.11 Tween 20

A-3.12 Bovine Serum Albumin

A-4 PROCEDURE

A-4.1 Preparation of Sample

A-4.1.1 Weigh 50 g of sample into blender jar.

A-4.1.2 Mix with 250 ml of 55 percent methanol and 45 percent water (*see* IS 1070).

A-4.1.3 Mix 100 ml hexane and blend for 1 min at high speed.

A-4.1.4 Filter mixture and recover filtrate.

A-4.1.5 Leave for 5 min and remove the lower phase containing methanol water (**A-4.1.2**).

A-4.2 Testing

A-4.2.1 Bring all reagents at room temperature (20-23°C).

A-4.2.2 Prepare fresh substrate in small culture tubes by mixing 500 µl substrate solution A with 500 µl substrate solution B for each reaction sites used.

A-4.2.3 Add 100 µl test extract to 200 µl buffer (**A-3.2**).

A-4.2.4 Thoroughly mix the diluted test extract and apply 100 µl diluted test extract to the centre of membrane. Using timer, wait for 1 min.

A-4.2.5 Apply 100 ml (2 drops) enzyme solution to the centre if membrane. Using timer, wait for 1 min.

A-4.2.6 Wash with 1.5 ml (30 drops) wash solution added drop wise.

A-4.2.7 Add the entire content of the substrate solution 1.0 ml from each test tube to each reaction site. Wait 1 min and immediately observe site (centre of cup) for blue colour development (negative) or no colour development (positive).

A-4.3 Interpretation of Results

A-4.3.1 Observe the reaction site (centre of the cup) for a blue colour or no colour development at exactly 1 min after adding the substrate A and B mixture (**A-3.3** and **A-3.4**).

Negative — If the reaction site (centre of the cup) turns light blue or darker, test sample contains total aflatoxin B1, B2 and G1.

Positive — If no blue colour is observed in the reaction site (centre of cup) and reaction site remains completely white (no colour change) for at least 1 min, the test sample contains aflatoxin B1, B2 and G1.

ANNEX B

[*Table* 1, *Sl No.* (x)]

DETERMINATION OF HEXANE RESIDUES IN OILS AND FATS

B-1 PRINCIPLE

The residual hexane content is the quantity of volatile hydrocarbons remaining in the fats and oils following processing involving the use of solvents. The volatile hydrocarbons are desorbed by heating the sample at 80°C in a closed vessel after addition of an internal standard. After determination of a calibration factor, hydrocarbons in the head space are determination of a calibration factor, hydrocarbons in the head space are determined by gas chromatography using packed or capillary columns. Results are expressed as hexane in mg/kg (or ppm). The method is applicable to the determination of 'free' volatile hydrocarbons expressed in terms of hexane remaining in animal and vegetable fats and oils after extraction with hydrocarbon based solvents. It is suitable for determination of quantities of hexane between 10 and 1 500 mg/kg in fats and oils.

B-2 APPARATUS

B-2.1 Gas Chromatograph

Gas chromatograph having

a) thermostatic column capable of maintaining the desired column temperature with in ± 1°C;
b) sample inlet system, separately thermostated which can be maintained at a minimum temperature of 100°C. If a capillary column is used, the inlet system must be capable of a 1/100 split injection. For serial analysis a headspace gas chromatograph with automatic sample injection and tempering bath is satisfactory; and
c) flame ionization detector which can be separately thermostated and maintained at a minimum of 100°C.

B-2.2 Recorder

If a recorder trace is to be used for calculating the composition of the samples analyzed, an electronic recorder of high precision is required or else use electronic integrator (**B-2.3**).

B-2.3 Electronic Integrator, which permits rapid and accurate calculations.

B-2.4 Chromatographic Column, either packed or capillary column with the following minimum requirements:

a) *Packed Column* — stainless steel or glass, approx 2 m long and 3.175 mm internal diameter with acid washed and silanized diatomaceous earth, 150-180 µm particle size (80-100 mesh Chromosorb WAW is suitable),

stationery phase — squalene consisting of 10 percent of packing.

b) *Capillary Column* — glass or fused silica approx 30 m long and 0.3 mm internal diameter.

Stationery phase — Methyl polysiloxane (film thickness 0.2 mm).

B-2.5 Syringe — 1 µl, 10 µ1, 1 000 µl capacity, gas tight.

B-2.6 Septum Vial — 20 ml capacity.

B-2.7 Septa and Aluminium Caps Suitable for Septum Vials Together with Crimping Pliers

The septa must be resistant to oils and solvents (butyl rubber or red rubber is recommended).

B-2.8 Tongs, suitable for holding septum vials.

B-2.9 Heating Bath, with clamps for holding septum vials, thermostatically regulated and capable of maintaining a temperature of 80°C. For continuous operation glycerol is recommended as heating liquid.

B-2.10 Shaking Machine

B-3 REAGENTS

B-3.1 Gases

a) *Carrier* — Helium (preferred for better resolution) or Nitrogen 99.99 percent pure, dried and containing a maximum of 10 mg O_2/kg.

b) *Flame Ionization Detector* — Hydrogen, minimum purity 99.95 percent, air or oxygen, dry, hydrocarbon free (less than 2 ppm hydrocarbon equivalent to CH_4).

B-3.2 Technical Hexane or Light Petroleum, with a composition similar to that used in industrial extraction or failing these *n*-hexane. For calibration, technical extraction hexane is preferred.

B-3.3 *n*-Heptane — (internal standard) analytical reagent grade.

B-3.4 Vegetable Oil — Solvent free, freshly refined and deodorized. The oil is to be used for calibration and should be of a similar nature as the sample. It should be free from extraction solvent (less than 0.01 percent).

B-4 SAMPLING AND SAMPLE STORAGE

It is essential that loss of solvent from the sample be prevented. The laboratory sample should be in a completely sealed condition and stored at 4°C. Plastic containers should not be used. Sample analysis should be carried out immediately when the sample container is opened.

B-5 GC OPERATING CONDITIONS

Carrier gas flow depends on the carrier gas and the type of column being used for analysis and should be optimized accordingly. The flow of hydrogen and air or oxygen to the FID should be optimized according to the manufacturer's recommendation. Injector and detector temperatures should be set at about 120°C. The column should be maintained at 40°C.

B-6 PROCEDURE

B-6.1 Determination of the Calibration Factor

Weigh to the nearest 0.01 g, 5 g of solvent free vegetable oil (**B-3.4**) into each of the 7 septum vials. Seal each vial with a septum and cap. By means of a syringe add technical Hexane to 6 of the seven vials (in the vial with no added solvent is the blank) according to the following table:

µl/5g	0.5	1	2	4	7	10
mg/100g	67	134	268	536	938	1 340

One vial remains without the addition of solvent.

If *n*-hexane is used for calibration the following table applies

µl/5g	0.5	1	2	4	7	10
mg/100g	66	132	264	528	924	1 320

Shake the 6 vials containing the solvent in the shaking machine vigorously for 1 h. Using the syringe add 5 µl of internal standard (**B-3.3**) to each of the 7 vials. Successively immerse the vials upto the neck in the heating bath at 80°C at intervals of approx 15 min. This time interval depends on the duration of the GC analysis which is complete on the elution of the internal standard (*n*-heptane). The samples must be placed in the heating at intervals such that each sample is tempered for exactly 60 min.

Warm the gas tight syringe to 60°C. After tempering at 80°C for exactly 60 min and without removing the vial from the heating bath, use the gas tight syringe and withdraw through the septum 1 000 µ1(1 ml) of the head space above the oil. inject immediately into the gas chromatograph. For each of the vial containing added solvent a calibration factor *F* may be determined by the following formula:

$$F = \frac{C_S \times A_1}{(A_H - A_B - A_1) \times C_1}$$

where

A_H = total peak area of solvent hydrocarbons including the area of internal standard present in the spiked oil. For identification purposes a typical chromatogram of solvent

composition should be obtained. Hydrocarbons which usually make up the technical hexane are 2 methyl pentane, 3 methyl pentane, methyl cyclo pentane, cyclohexane etc. Do not include peaks due to oxidation products which may be present in significant amounts.

A_B = peak area of the solvent hydrocarbons present in the oil to which solvent has not been added (blank) less the peak are of the internal standard.

A_1 = peak area corresponding to the internal standard in the spiked samples.

C_1 = quantity of the internal standard added expressed in mg/kg of the oil.

C_s = quantity of technical hexane added to the oil present in the vial expressed in mg/kg of the oil.

Express the results to the third decimal place.

Calibration factors of the six standards should be approximately the same. The mean calibration factor should be 0.45 if *n*-heptane is used and 0.57 if cyclohexane is used.

The factor (*F*) so evaluated can be used for determining vial quantities of hexane less than 60 mg/kg. If the value of *F* found for the vial containing 0.5 µl of hexane is significantly below the mean value, this deviation is probably due to difficulty in introducing exactly 0.5 µl and this determination must be either eliminated or repeated. For quantities of hexane between 10 and 20 mg/kg it is better to prepare calibration standards by adding 2 µl of internal standard instead of 0.5 µl.

B-6.2 Sample Analysis

Weigh to the nearest 0.01 g, 5 g of the test sample into a septum vial as quickly as possible and close immediately with a septum and cap. Using a syringe add through the septum exactly 5 µl of the internal standard. Shake vigorously by hand for about 1 min and then immerse the vial upto the neck in the heating bath. At 80°C for exactly 60 min. Warm the gas tight syringe to 60°C. After tempering at 80°C for exactly 60 min use the gas tight syringe and take from the vial without removing it from the bath 1 000 µl (1 ml) of the head space above the sample. Immediately inject into the gas chromatograph. Carry out two determinations in rapid succession on each sample.

B-7 CALCULATION

The residual solvent expressed in mg/kg (ppm) is given by the following formula:

$$W = \frac{(A_H - A_1) \times F \times C_1}{A_1}$$

where

A_H = total peak area of solvent hydrocarbons including the area of internal standard. Hydrocarbons which usually make up the technical solvents are 2 methyl pentane, 3 methyl pentane, methyl cyclopentane, cyclohexane etc. Do not include peaks due to the oxidation products. Some of these products may be present in significant amount.

A_1 = peak area corresponding to internal standard in the sample.

C_1 = quantity of the internal standard added in mg/kg.

NOTE — For an addition of 5 µl of heptane/5 g of sample C_1 = 680 mg/kg and C_1 = 750 mg/kg if cyclohexane is used.

F = calibration factor obtained in procedure

Report as the final result the mean of the results of two determinations.

ANNEX C

[*Table* 1, *Sl No.* (xi)]

DETERMINATION OF PHOSPHOROUS

C-1 PRINCIPLE

The test portion is ignited and the ashes so obtained are treated with nitric acid. Ammonium molybdate reacts with phosphorous under acidic conditions to form molybdophosphoric acid, which in presence of vanadium forms yellow phosphovanadomolybdic complex. The intensity of yellow colour is proportional to phosphate concentration.

C-2 APPARATUS

C-2.1 Calibrated Pipettes, 5 ml and 20 ml.

C-2.2 Crucible or Porcelain Evaporating Dish, 40-50 ml.

C-2.3 Colorimeter or Spectrophotometer, suitable for observations at 460 nm.

C-2.4 Furnace, giving a temperature of 800 to 900°C.

C-3 REAGENTS

C-3.1 Magnesium Oxide, light, pure, free from phosphorus.

C-3.2 Nitric Acid, approximately 6N aqueous solutions.

C-3.3 Ammonium Molybdate [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$], analytical reagent quality, 50 g/1 aqueous solution.

C-3.4 Acid Aqueous Solution of Ammonium Vanadate [NH_4VO_3]

Dissolve about 2.5 g of ammonium vanadate in about 500 ml of hot water. Cool, and then add about 20 ml of nitric acid (ρ = 1.33g/ml). Make up to 1 litre with distilled water (*see* IS 1070).

C-3.5 Standardized Solution Containing 1 mg of Phosphorus per ml

Dissolve 1.156 (± 0.001) g of disodium hydrogen phosphate [Na_2HPO_4, 12 H_2O], or 0.439 3 (± 0.000 5) g of mono-potassium hydrogen phosphate [KH_2PO_4] in exactly 100 ml of distilled water (*see* IS 1070).

C-4 PROCEDURE

C-4.1 Calibration

Dilute the standardized solution (**C-3.5**) in such a mane as to obtain solutions containing exactly 0.4, 0.2, 0.1, 0.05, and 0.01 mg of phosphorus per ml. Utilize these solutions in order to plot the calibration curve of the instrument (**C-2.3**) in such a way that this curve expresses the results in mg of phosphorus per ml of the final solution.

C-4.2 Determination

Weigh to within 1 mg exactly 0.1 g of magnesium oxide (**C-3.1**) into the crucible or porcelain dish (**C-2.2**), and ignite. Allow to cool, then weigh, to within 1 mg about 0.1 to 10 g of fat, according to its presumed phosphorus content. Burn of the fat (if necessary with the assistance of a folded, ash less filter paper). Ignite to a white ash in the furnace (**C-2.4**) at 800 to 900°C. Dissolve the magnesium containing ash in exactly 5 ml of the aqueous nitric acid solution (**C 3.2**) with the aid of a 5 µl pipette (**C-2.1**). Add exactly 20 ml of a mixture of 10 ml of the aqueous ammonium molybdate solution (**C-3.3**) and 10 ml of the acid aqueous ammonium vanadate solution (**C-3.4**). Mix and allow to stand for 20 min. Prepare a blank test, not containing fat, under exactly the same conditions. Transfer the test solution into the cell of the apparatus (**C-2.3**), Measure the extinction at 460 nm against the blank solution. Read the absorbance (or other indications on the scale of the colorimeter).

C-5 EXPRESSION OF RESULTS

With the aid of the calibration curve obtained according to **C-4.1** and absorbance or other figure read on the instrument, read from the graph the amount of phosphorus (m_1) in mg per ml of test solution.

The percentage (*m*/*m*) of phosphorus present in the oil is given by the following formula:

$$\text{Phosphorous content (percent)} = \frac{2.5\, m_1}{m}$$

where

m_1 = concentration of phosphorus read from the curve, in mg per ml; and

m = mass of the test portion, in g.

ANNEX D

[*Table* 1, *Sl No.* (xii)]

TEST FOR INSOLUBLE BROMIDES

D-1 PROCEDURE

Dissolve in a test tube about 6 g of the material in 12 ml of a mixture of equal parts of chloroform and acetic acid. Add bromine, dropwise, until a slight excess is indicated by the colour, keeping the solution at 20°C. Allow the mixture to stand for at least 15 min and place the test tube in boiling water.

D-2 RESULT

The material shall be taken to have passed the test if the solution when kept in boiling water, does not become cloudy on account of insoluble bromides.

Bureau of Indian Standards

BIS is a statutory institution established under the *Bureau of Indian Standards Act*, 1986 to promote harmonious development of the activities of standardization, marking and quality certification of goods and attending to connected matters in the country.

Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards : Monthly Additions'.

This Indian Standard has been developed from Doc No.: FAD 13 (2432).

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

BUREAU OF INDIAN STANDARDS

Headquarters:

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110002
Telephones : 2323 0131, 2323 3375, 2323 9402 *Website*: www.bis.org.in

Regional Offices: *Telephones*

Central : Manak Bhavan, 9 Bahadur Shah Zafar Marg, NEW DELHI 110002 — 2323 7617, 2323 3841

Eastern : 1/14 C.I.T. Scheme VII M, V. I. P. Road, Kankurgachi, KOLKATA 700054 — 2337 8499, 2337 8561, 2337 8626, 2337 9120

Northern : SCO 335-336, Sector 34-A, CHANDIGARH 160022 — 260 3843, 260 9285

Southern : C.I.T. Campus, IV Cross Road, CHENNAI 600113 — 2254 1216, 2254 1442, 2254 2519, 2254 2315

Western : Manakalaya, E9 MIDC, Marol, Andheri (East), MUMBAI 400093 — 2832 9295, 2832 7858, 2832 7891, 2832 7892

Branches: AHMEDABAD. BANGALORE. BHOPAL. BHUBANESHWAR. COIMBATORE. DEHRADUN. FARIDABAD. GHAZIABAD. GUWAHATI. HYDERABAD. JAIPUR. KOCHI. LUCKNOW. NAGPUR. PARWANOO. PATNA. PUNE. RAJKOT. VISAKHAPATNAM.

Published by BIS, New Delhi